Anti-Inflammatory Effect of Cabbage Juice Mixed with Honey on Gastric Ulcer in Experimental Rats.

Okorie, A. C., Kanu, A. M. and Ihemanma, C. A.
Department of Biology Microbiology, Abia State Polytechnic Aba.
Corresponding author: chukwuemeka. Okorie@abiastatepolytechnic.edu.ng
Phone:080333 74735

Honey is substance produced by bees from the nectar of plants. It is used as a medicine and known for its biological properties, having antibacterial, antifungal and healing properties. While *Brassica oleracea* was cultivated from ancient times and used as food and in herbal medicine. It has been well known that cabbage juice can reduce constipation and has also been used as laxative, as an antidote to mushroom poisoning, or a treatment of hangovers and headache. This work was designed to explore the anti-inflammatory effect of Cabbage juice mixed with Honey on the healing of gastric ulcer in experimental rats. Thirty albino rats (100 + 5g) were used and allocated into 6 equal groups. Group 1 was used as negative control while group 2-5 were induced with ethanol (1ml) orally. Results revealed that treatment with cabbage juice mixed with honey significantly inhibited degree of ulcerations in rats when compared with ulcer control. The mean ulcer score in the ulcer control was 33.90 ± 4.99 while in the group treated with 20ml/kg of cabbage leaf juice, ulcer score was 18.80 ± 1.92. Ulcer score in the 20ml/kg honey treated group was 13.20 ± 5.06 while a combination of both the juice and honey yielded an ulcer score of 15.80 ± 1.82. Ulcer score in groups treated with both the cabbage leaf juice and honey were significantly lower than the mean score obtained in Omeprazole treated group ($P < 0.05$) Ulcer indices were also significantly lower in the cabbage leaf juice and honey treated rats when compared with ulcer control group ($P < 0.05$). It is concluded that Cabbage juice mixed with Honey could be used for healing of gastric ulcer.

Keywords: Honey, Cabbage, Omeprazole, Rats

Introduction

Gastric Ulcer is a major gastrointestinal disease constituting an important health issue in all societies Gastric ulcers are a break in the mucosa of the stomach lining that penetrates through the muscular mucosa and extends more than 5mm in diameter (Kayali, et al. 2018 & Mating and Nwaolo 2014). When alterations occur to the defense mechanisms of the stomach, it can cause changes in the gastric mucosa which will eventually result in erosion and then ulceration. Cabbage (*Brassica Oleracea Var Capitata*) is a small leafy biennial producing a compact globular mass of smooth or crinkle leaves wrapped over each other known as head. The outer leaves are generally larger than the inner. The stem is short and stout leaves are low in calories (27%), Fat (0.1%) and Carbohydrates (4.6%) (Ali and Al-Swayeh 2023). It is good source of protein (1.3%) which contains all essential amino acids particularly Sulphur containing amino acids (Loana and Andrea., 2020). Cabbage is an excellent source of minerals such as Calcium (39mg), Iron (0.8mg), Magnesium (10mg), Sodium (14.1mg), Potassium (114mg) and Phosphorus (44mg) It has substantial amounts of B. Carotene ascorbic acid, riboflavin, niacin and thiamins (Al-mazrooa et al. 1999).

Cabbage is a popular natural ulcer remedy. Doctors reportedly used it decades before antibiotics were available to help stomach ulcers. Its rich in Vitamin C, an antioxidant shown to help prevent and treat H. pylori infections (Barchi et al 2011 and Bukhari et al 2011). These

infections are the most common cause of Stomach Ulcers (Jarosz, *et al.,* 1998 and Tabak, *et al.,* 2003). In fact, several animal studies show that cabbage juice is effective at treating and preventing a wide range of digestive ulcers, including those affecting the stomach (Taibi, *et al.* 2014).

Honey is a powerful antibacterial and has been shown to inhibit *H. pylori* growth. As long as you would any sweetener with the bonus of perhaps soothing your ulcers. Honey is one of the most popular medicinal ingredients used in Ayurvedic system of medicine. It is good for many stomach conditions such as gastric and peptic ulcers. It is mainly recommended to manage stomach ulcers. Honey has healing powers that might be beneficial in dealing with stomach ulcers (Muriel et al, 2017 and Pillai, et al. 2010). Finally, the researcher believes that combination of cabbage and honey will be effective than when one is used.

Materials and Method

Location of the Project

The research was carried out at the HND II laboratory at Abia state polytechnic, Aba, Abia State. Abia State lies within approximately latitude $4^0$40 and $6^0$14' North and Longitude $7^0$10 and 80 East. It covers an area of about 5,833,999 million people.

Experimental Animals

Thirty (30) mature wistar rats was used for the study. The animals were procured from Michael Okpara University of Agriculture, Umudike in wire mesh rat cages and allowed free access to pelleted feed (Vital feed, Nigeria) and clean water, except when fasting is required.

Plant Collection and Extract Preparation

The fresh leaves of cabbage were purchased from New market in Aba. The botanical identification and authentication were confirmed at the department of Biology / Microbiology by a botanist. The leaves were washed properly. The fresh leaves were crushed and refluxed for an hour concentrated to on tenth ($^1/_{10}$) of the original volume at 38 – 40^0C using rotary evaporator.

Honey Solution

Pure unprocessed, non – boiled commercial wild honey was obtained from Nsukka for this study. Honey was examined, chemically tested and found to be free from inverted sugar, liquid, glucose or dextran. For experiments, fresh 10% w/v solution was prepared with distilled water.

Experimental Design

3Acute toxicity test.

Thirty-five mature rats of either sexes were randomly assigned to 7 groups of 5 rats each and fasted for 12 hours. One group served as control while the remaining 6 groups were treated orally with varying doses (500-5000 mg/kg) of MECO. The rats were observed for 24 hours for signs of acute toxicity and death.

Diclofenac-induced ulcer

Model 1 – Single dosing. Thirty-six rats were randomly assigned to 6 groups (n = 6), fasted for 48 hours and treated as follows:

Group I	Normal animals (no treatment)
Group II	Distilled water/vehicle (5 ml/kg)
Group III	Diclofenac (100 mg/kg) only
Group IV	Diclofenac (100 mg/kg) + MECO (low dose)
Group V	Diclofenac (100 mg/kg) + MECO (medium dose)

Group VI Diclofenac (100 mg/kg) + MECO (high dose)

All treatments were done orally by means of gastric gavage. The extract was given 1 hour before administration of the ulcerogenic agent (diclofenac). Six hours later, all the rats were sacrificed by cervical dislocation, the stomach incised along the greater curvature and washed with ice cold normal saline. The gastric mucosa will be observed for ulcer lesions, using a magnifying glass. Determination of ulcer index will be done as follows:

$U_I = (U_N + U_S + U_P) \times 1/10$

Where U_I = ulcer index; U_N = mean number of ulcer per animal; U_S = mean severity score; U_P = percentage of animals with ulcer.

The percentage ulcer inhibition will be calculated using the formula:

PUI = UI (control) – UI (treated) / UI (control) x 100.

Collection of gastric juice

The juice collected was centrifuged at 60 rpm for 10minutes and the volume of gastric juice was measured. The gastric juice was used for biochemical estimation.

Determination of free acidity and total acidity

Gastric juice (1ml) was taken into a 100ml conical flask, to this 2-3 drops of Topfer's reagent was added and titrated with 0.01N NaOH until all traces of red colour disappears and the colour of the solution turns yellow orange (end point). The volume of alkali added was noted. This volume corresponds to free acidity. 2-3 drops of phenolphthalein solution were added and titration was continued until a definite red tinge reappears. The volume of alkali was noted which corresponds to total acidity.

Calculations:

Acidity was calculated by using the formula:

$$\text{Acidity (mEq/litre)} = \frac{\text{Volume fo NaOH x Normality of NaOH x 100}}{0.1}$$

Estimation of pepsin activity

For pepsin estimation, 4 placed test tubes (1) and (2) containing 5ml of 1% bovine albumin in 0.01M Hcl, (3) and (4) containing 10Ml of 0.35 M trichloroacetic acid. The gastric juice was mixed with an equal volume of 0.01 M Hcl warmed to 37^{o}C. 1ml of this mixture was added to each of the test tubes of (1) and (4) incubated for 15 minutes. At the end, the mixed content of tube (1) with (3) were allowed to stand for about 4 minutes. (1) + 3 give test and (2) + (4) give blank. The mixture was filtered. To 2ml of filtrate, 10ml of NaOH was added. Then 1ml of phenol reagent was added and mixed by gentle rotation. After 30 minutes, the absorbance was measured at 680nm. The difference between test and blank give the measures of peptic acidity.

pH of the gastric juice

pH of the gastric juice was determined by end point filtration at pH 7:00 using as titrant of sodium hyudroxide solution of 0.71 equivalent/litre (Ghosh *et al.*, 2011).

Determination of Enzymatic Antioxidants

Estimation of superoxide dismutase (SOD)

Superoxide dismutase activity was assayed by the method of Arthur and Boyne (1985) as contained in Randox kit.

Principle: The method employs xanthine and xanthine oxidase to generate superoxide radicals which reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD causes a 50% inhibition rate in the reduction of (I.N.T) under the conditions of the assay.

Procedure: To 0.05ml diluted sample in a test tube was added 1.7ml mixed substrate solution and mixed xanthine oxidase (0.25ml) was added and the initial absorbance taken after 30 seconds. The final absorbance was taken after 3 minutes and units of SOD per gram haemoglobin were extrapolated from a standard curve.

Estimation of catalase

The activity of catalase was assayed by the method of Sinha (1972).

Principle: Dichromate in acetic acid was reduced to chromic acetates, when heated in presence of hydrogen peroxide with the formation of per chronic acid as unstable intermediate. The chronic acetate formed was measured at 570nm. Catalase was allowed to split H_2O_2 for different periods of time. The reaction was stopped at different time intervals by the addition of dichromate acetic acid mixture in 1:3 and the remaining H_2O_2 was determined by measuring chromic acetate colorimetrically.

Procedure: To 0.9ml of distilled water and 0.1ml of plasma in a test tube was added 2ml of H_2O_2 and 2ml phosphate buffer. The reaction was initiated by added 2ml of dichromate acetic acid reagent to 1ml portion to this mixture. Absorbance of the reaction was taken in 30 seconds interval for 2 minutes. The activity of catalase was expressed as U/ml of plasma (U-micro-moles of H_2O_2 utilized per second.

Determination of Liver function parameters

At the end of the treatment period, all the animals were sacrificed and blood was collected by cardiac puncture into plane tubes, which after clotting and retraction were centrifuged using a bench centrifuge to obtained clear sera which were used for assaying some liver function parameters including;

i. Alanine aminotransferase (ALT),
ii. Aspartate aminotransferase (AST)
iii. Alkaline phosphatase (ALP)
iv. Total bilirubin
v. Total protein

Determination of haematological parameters

Haematological analysis of the blood samples was performed in an automated haematology analyzer (BC-2300 model, Mindray Medical Co., China) with the procedure carried as specified by the producer. The parameters which were evaluated included: red blood cells count(RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); platelets (PLT); total leukocytes count (TLC) count and differential leukocytes count(WBC) counts were obtained at once for each blood sample.

Principles of Haematological Analyzer

The haematological parameters were determined using the automated haematology analyzer according to the methods by Dacie and Lewis (1991), modified by Chabra, (2018) and Bain *et al., (*2012) following the procedure outlined by the producer.

Determination of renal function parameters

Urea

The contents of vial R1a were transferred into bottle R1b and mix gently forming reagent R1. Also, the contents of bottle R2 was diluted with 660 ml of distilled water and mix thoroughly. Also, the contents of bottle R3 was diluted with 750 ml of distilled water. The test was then ready to start. Three test tubes labelled test, blank and standard were set up. Approximately 100 µl of R1 was pipetted into each of the test tubes and 10µl of the test sample added into the test tube labelled test. The same volume of the standard reagent and distilled water were added to the test tubes labelled standard and blank respectively. The mixtures in the test tubes were incubated at 37^0C for 10 minutes and thereafter 2.5 ml of reagent R2 and the same volume of R3 added to each test tube. The contents of each tube were mixed immediately and incubated at 37^0C for 15 minutes. At the end, the absorbance of the test and that of the sample were read after zeroing with blank. The urea concentration in the sample was calculated thus:

$$\text{Urea concentration in mg/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

Where concentration of standard = 78.73 mg/dl

Results

Table 1a: Stage 1 acute toxicity (LD_{50}) of Cabbage leaf juice

Group	Dose (ml/kg)	No. of Deaths	Percentage of mortality	Observations
1	5	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
2	10	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
3	15	0/3	0.00	No mortality observed, instead animals remained active and physically stable

Table 1b: Stage 2 acute toxicity (LD_{50}) of Cabbage leaf juice

Group	Dose (ml/kg)	No. of Deaths	Percentage of mortality	Observations
1	16	0/3	0.00	No mortality observed, instead animals remained active and physically stable
2	29	0/3	0.00	No mortality observed. Animals were initially calm but regained physical activity within one hour of administration.
3	50	0/3	0.00	No mortality observed. Animals were initially calm and depressed but regained physical activity within 24 hours of administration.

LD_{50} is greater than 50 ml/kg body weight

Results of acute toxicity evaluation of Honey

Table 1c: Stage 2 acute toxicity (LD_{50}) of Honey

Group	Dose (ml/kg)	No. of Deaths	Percentage of mortality	Observations
1	5	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
2	10	0/3	0.00	No mortality observed, instead animals remained active and physically stable
3	15	0/3	0.00	No mortality observed, instead animals remained active and physically stable

Table 1d: Stage 2 acute toxicity (LD_{50}) of Honey

Group	Dose (ml/kg)	No. of Deaths	Percentage of mortality	Observations
1	16	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
2	29	0/3	0.00	No mortality observed. Animals were initially calm but regained physical activity within one hour of administration.
3	50	0/3	0.00	No mortality observed. Animals were initially calm and depressed but regained physical activity within 24 hours of administration.

LD_{50} is greater than 50 ml/kg body weight

Table 2: Anti-inflammatory effects of cabbage leaf juice and honey against ethanol-induced stomach ulcers in rats

Treatment	U_N	U_S	U_P	Ulcer index (U_I)	Percentage ulcer inhibition
Normal control	0.00a	0.00^{a}	0.00^{a}	0.00^{a}	100.00 ± 00^{e}
Ulcer control	10.00 ± 1.58^{d}	33.90 ± 4.99^{e}	100.00 ± 0.00^{e}	14.39 ± 0.65^{e}	0.00^{a}
Omeprazole, 20 mg/kg	8.20 ± 0.84^{c}	26.10 ± 2.38^{d}	93.00 ± 2.74^{d}	12.72 ± 0.51^{d}	9.71 ± 3.46^{b}
Cabbage leaf juice 20 ml/kg	8.20 ± 0.8^{c}	18.80 ± 1.92^{c}	92.00 ± 2.74^{cd}	11.90 ± 0.46^{c}	15.75 ± 3.41^{c}
Honey, 20 ml/kg	4.80 ± 0.84^{b}	13.20 ± 5.06^{b}	84.00 ± 5.48^{b}	10.10 ± 1.07^{b}	28.44 ± 7.60^{d}
Cabbage leaf juice + Honey (20 ml/kg)	6.80 ± 1.30^{c}	15.80 ± 1.82^{bc}	89.00 ± 2.24^{c}	11.16 ± 0.51^{c}	20.85 ± 3.61^{c}

Values are presented as mean ± standard deviation of replicated determination (n = 5).Means in the same column bearing different letter superscripts are statistically significantly different.

Table 3: Effect of cabbage leaf juice and wild honey on extractible mucus weight, total acidity and pepsin activity in ethanol-induced ulcerated rats

Treatment	Extractible mucus weight (g)	Pepsin activity (µmol tyrosine/ml)	Total acidity (mmol/L)	pH
Normal control	0.04±0.00^{f}	17.60±1.62^{a}	89.88±2.99^{a}	2.90±0.04^{cd}
Ulcer control	0.01±0.00^{a}	30.20±1.84^{e}	118.22±2.01^{d}	2.38±0.05^{bc}
Omeprazole, 20 mg/kg	0.02±0.00^{b}	25.24±0.64^{d}	106.00±3.35^{c}	2.85±0.13^{b}
Cabbage leaf juice 20 ml/kg	0.02±0.00^{c}	23.62±1.12^{cd}	96.64±1.92^{b}	2.99±0.04^{cd}
Honey, 20 ml/kg	0.03±0.00^{e}	21.96±1.57^{bc}	94.90±1.77^{b}	3.03±0.06^{d}
Cabbage leaf juice + Honey (20 ml/kg)	0.03±0.00^{d}	20.94±1.23^{b}	96.56±2.12^{b}	2.99±0.11^{cd}

Values are presented as mean ± standard deviation of replicated determination (n = 5). Means in the same column bearing different letter superscripts are statistically significantly different.

Table 4 Changes in haematological values in ethanol-induced rats pretreated with cabbage leaf juice and wild honey

Treatment	RBC (x10^{6}/mm^{3})	PCV (%)	Hb (g/dl)	WBC (x10^{3}/mm^{3})	PLT (x10^{3}/mm^{3})	MCV (fl)	MCH (pg)	MCHC (g/dl)
Normal control	7.03± 0.16^{ab}	44.40± 1.14^{a}	15.62± 0.53^{a}	8.92± 0.25^{a}	245.00±3 .94^{a}	63.14± 0.61b	22.21± 0.34^{a}	35.17± 0.34^{ab}
Ulcer control	7.68± 0.22^{c}	47.00± 1.00^{c}	17.36± 0.49^{b}	13.75± 1.12^{d}	255.20± 9.26^{b}	61.24±0. 63^{a}	22.62±0. 25^{abc}	36.94±0.62^{c}
Omeprazole, 20 mg/kg	7.19± 0.19^{b}	46.20± 1.30^{b}	16.18± 0.50^{a}	11.70± 0.91^{c}	249.80± 4^{ab}.21	64.27± 0.36^{ab}	22.51± 0.37^{ab}	35.02± 0.56^{a}
Cabbage leaf juice 20 ml/kg	7.13± 0. 06^{ab}	46.20± 0.44^{bc}	16.20± 0.19^{a}	10.58± 0.76^{b}	247.60±4 .88^{a}	64.82±0. 52^{c}	22.73±0. 30^{abc}	35.07± 0.66^{a}
Honey, 20 ml/kg	6.94± 0.18^{a}	44.60± 0.55a	16.00± 0.35^{a}	9.89± 0.51^{b}	245.20 ±4.60^{a}	64.31± 1.44^{ab}	23.07± 0.33^{c}	35.87± 0.61^{b}
Cabbage leaf juice + Honey (20 ml/kg)	7.12± 0. 15^{ab}	45.20± 0.84^{ab}	16.22±0 .41^{a}	10.23 ±0.35^{b}	245.00± 2.92^{a}	63.53± 1.22^{b}	22.80± 0.57^{bc}	35.88± 0.40^{b}

Values are presented as mean ± standard deviation of replicated determination (n = 5). Means in the same column bearing different letter superscripts are statistically significantly different.

Table 5: Effect of cabbage leaf juice and wild honey on liver function parameters in ethanol-induced ulcerated rats

Treatment	Total protein (g/dl)	ALT (u/l)	AST (u/l)	ALP (u/l)	Bilirubin (mg/dl)
Normal control	5.91±0.24^{a}	24.20±2.95^{a}	35.80±1.30^{a}	83.60±3.91^{a}	0.66±0.03^{a}
Ulcer control	5.86±0.29^{a}	46.40±1.67^{d}	60.20±4.21^{d}	88.60±3.58^{a}	0.93±0.04^{d}
Omeprazole, 20 mg/kg	6.02±0.66^{a}	42.40±2.88^{c}	51.40±2.79^{c}	83.60±5.94^{a}	0.86±0.05^{c}
Cabbage leaf juice 20 ml/kg	6.21±0.29^{a}	39.40±1.67^{c}	45.40±1.14^{b}	84.60±1.52^{a}	0.78±0.06^{b}
Honey, 20 ml/kg	5.96±0.15^{a}	35.00±2.12^{b}	43.00±2.12^{b}	85.00±1.00^{a}	0.74±0.03^{b}
Cabbage leaf juice + Honey (20 ml/kg)	6.08±0.17^{a}	33.60±2.51^{b}	45.00±3.61^{b}	86.60±2.07^{a}	0.80±0.03^{b}

Values are presented as mean ± standard deviation of replicated determination (n = 5). Means in the same column bearing different letter superscripts are statistically significantly different.

Table 6: Effect of cabbage leaf juice and wild honey on renal function parameters in ethanol-induced ulcerated rats

Treatment	Urea (mg/dl)	Creatinine (mg/dl)	Na^{+} (mEq/L)	K^{+} (mEq/L)	Cl^{-} (mEq/L)	HCO_3^{-} (mmol/L)
Normal control	18.73±0.52^{a}	0.67±0.02^{a}	132.30±1.50^{a}	4.41±0.03^{a}	88.74±1.31^{a}	19.66±0.4^{a}
Ulcer control	23.62±1.73^{d}	0.87±0.03^{e}	142.38±3.58^{c}	4.69±0.06^{c}	93.38±0.97^{c}	20.72±0.34^{c}
Omeprazole, 20 mg/kg	21.96±0.83^{c}	0.77±0.05^{d}	138.12±2.85^{b}	4.47±0.07^{ab}	91.42±0.78^{b}	20.54±0.23^{c}
Cabbage leaf juice 20 ml/kg	21.38±0.43^{c}	0.72±0.01^{bc}	136.04±1.86^{b}	4.47±0.06^{ab}	90.24±1.30^{b}	20.14±0.15^{b}
Honey, 20 ml/kg	19.92±0.54^{a}	0.71±0.03^{ab}	135.080.85^{ab}	4.54±0.12^{b}	90.06±0.54^{b}	20.06±0.40^{b}
Cabbage leaf juice + Honey (20 ml/kg)	20.88±1.30^{bc}	0.76±0.03^{cd}	134.90±2.71^{ab}	4.48±0.101.31^{ab}	90.32±0.70^{b}	20.08±0.13^{b}

Values are presented as mean ± standard deviation of replicated determination (n = 5). Means in the same column bearing different letter superscripts are statistically significantly different.

Table 7: Effects of cabbage leaf juice and wild honey on serum antioxidant parameters in ethanol-induced ulcerated rats

Treatment	GSH (mg/dl)	GPx (u/l)	SOD (u/l)	CAT (u/l)	MDA (mmol/L)
Normal control	9.53±0.47^{e}	45.08±3.85^{d}	37.18±1.54^{a}	27.32±1.17^{c}	0.46±0.03^{a}
Ulcer control	6.94±0.26^{a}	34.88±1.34^{a}	32.36±1.89^{a}	21.72±1.45^{a}	1.28±0.16^{c}
Omeprazole, 20 mg/kg	7.45±0.21^{b}	37.28±1.39^{ab}	35.38±1.83^{a}	24.08±0.62^{b}	0.81±0.0^{b}
Cabbage leaf juice 20 ml/kg	8.60±0.40^{cd}	41.66±4.48^{cd}	35.60±1.42^{a}	24.82±0.87^{b}	0.800.08^{b}
Honey, 20 ml/kg	8.94±0.22^{d}	40.44±3.49^{bc}	37.38±1.78^{a}	25.20±1.08^{b}	0.72±0.02^{b}
Cabbage leaf juice + Honey (20 ml/kg)	8.26±0.21^{c}	41.50±1.581.54^{cd}	36.44±2.15^{a}	24.30±0.64^{b}	0.75±0.06^{b}

Values are presented as mean ± standard deviation of replicated determination (n = 5). Means in the same column bearing different letter superscripts are statistically significantly different.

Statistical Analysis

The data obtained from the study were subjected to One-Way analysis of Variance (ANOVA). The means were compared using a Duncan multiple range comparison test with level of statistical significance established at 95% confidence interval. The statistical analysis was performed using software: Statistical Products and Service Solutions (SPSS) version 23.

Discussion

Stomach ulcers (Gastric ulcers) are open sores that develop on the lining of the stomach. Ulcers can also occur in part of the intestine just beyond the stomach. Graded doses up to 50 ml/kg of Cabbage leaf juice and Honey produced no mortality in rats. The rats administered up to dose remained active and physically stable throughout the 12 hours period. Toxicity signs such as agitation, roughness of hairs, writhing reflexes, calmness, convulsions, tremor and loss of motor coordination were also not observed.

Ethanol successfully induced ulcer in the test rats when compared with the normal control group. However, treatment with cabbage leaf juice and Honey significantly inhibited the degree of ulceration in the rats when compared with the ulcer control group. This agrees with the work of Agrawal et al.,2000.

Graded doses up to 50 ml/kg of honey produced no mortality in rats. The rats administered up to this dose remained active and physically stable throughout the 24-hour period and a further seven days of observation. Toxicity signs such as agitation, roughness of hairs, writhing reflexes, calmness, convulsions, tremor and loss of motor coordination were also not observed. Therefore, the LD_{50} of the leaf juice was therefore established to be greater than 50 ml/kg body weight. These results are presented in tables 4.1c and 4.1d.

Ethanol successfully induced ulcer in the test rats when compared with the normal control group ($p<0.05$). However, treatment with cabbage leaf juice and also honey significantly inhibited the degree of ulcerations in the rats when compared with the ulcer control group. For example, mean ulcer score in the ulcer control group was 33.90±4.99 while in group treated with 20 ml/kg of cabbage leaf juice, ulcer score was 18.80±1.92. Ulcer score in the 20 ml/kg honey treated group was 13.20±5.06 while a combination of both the juice and honey yielded an ulcer score of 15.80±1.82. Ulcer scores in groups treated with both the cabbage leaf juice and honey were significantly lower than the mean score obtained in the omeprazole treated group ($p<0.05$). Ulcer indices were also significantly lower in the cabbage leaf juice and honey treated rats when compared with ulcer control group ($p<0.05$). Ulcer indices in the cabbage leaf juice and honey treated rats were 11.90±0.46 and 10.10±1.07 respectively, both being significantly lower than ulcer index of 12.72±0.51obtained for the ulcer control group (Table 4.2). This also agrees with the work of Agrawal, et al. 2002.

Percentage inhibition of ulcer in the juice treated rats was 15.75±3.41% while in the group treated with honey, percentage ulcer inhibition was 28.44±7.60%. Cabbage leaf juice and honey when combined together produced an ulcer inhibition of 20.85±3.61% (Table 4.2 and fig. 4.1). This agrees on the work of Agrawal, et al. 2002.Red blood cells count, PCV values and haemoglobin concentration were significantly higher in the ulcerated rats when compared with normal control ($p<0.05$), but values of these parameters significantly lowered towards normal values in groups treated with cabbage leaf juice, honey and their combined form. For example, RBC count was 7.03±0.16 $x10^6/mm^3$ in the normal control group, but increased to 7.68 ±0.22 $x10^6/mm^3$ in the ulcer control group. Values of RBC obtained for the groups treated with cabbage leaf juice and wild honey were 7.13±0.06 $x10^6/mm^3$ and 6.94 ±0.18 $x10^6/mm^3$ respectively (Table 4.4).

RBC count in group treated with the juice and honey in combined form had7.12±0.15 $x10^6/mm^3$. Results of PCV and haemoglobin concentration followed same pattern as those obtained for RBC (Table 4.4). Total white blood cells count and platelets count were both significantly increased in the ulcerated rats when compared with normal control but the treatment agents reduced the values towards the direction of the normal. Values of MCV, MCH and MCHC were not so significantly altered ($p>0.05$), except that mean value of MCV in the ulcer control group was significantly lower than that of the normal control and other test groups (Table 4.4 and fig. 4.3). Values of renal function parameters including urea, creatinine and the electrolytes were all significantly increased following induction of ulcer with ethanol when the values obtained for the induced groups were compared with those obtained in the normal control group ($p<0.05$). Urea concentration in the normal control group was 18.73±0.52 mg/dl but increased to 23.62±1.73 mg/dl in the ulcer control group, but significantly reduced to 21.96±0.83 mg/dl inthe Omeprazole treated group, 21.38±0.43 mg/dl in the cabbage leaf juice treatment group,19.92±0.54 mg/dl in the wild honey treated group and 20.88±1.30 mg/dl in the group treated with both the juice and wild honey (Table 4.6).

Similar pattern of results was obtained for serum creatinine concentration. Serum electrolytes including sodium, potassium, chloride and bicarbonate values were also significantly higher in the induced rats when compared with the normal control ($p<0.05$), but were all also significantly lowered in groups treated with cabbage juice, wild honey and a combination of both (Table 4.6 and Fig.4.5).

Reduced glutathione (GSH) concentration was significantly lower in the ulcer control group when compared with normal control ($p<0.05$). GSH concentration in the normal control was 9.53±0.47 mg/dl but reduced to 6.94±0.26 mg/dl in the ulcer control group. Pretreatment with the test's agents (cabbage leaf juice and wild honey) significantly inhibited the observed fall in GSH concentration due to ulcer induction when also compared with the ulcer control group ($p<0.05$) and compared favourably with the activity of the standard drug, Omeprazole. For example, GSH concentration in group treated with the juice and wild honey were 8.60±0.40 mg/dl and 8.94±0.22 mg/dl respectively, while the group treated with combine juice and honey had 8.26±0.21 mg/dl as serum GSH concentration (Table 4.7). Glutathione peroxidase (GPx) activities were also significantly lower in the ulcer control group than the normal control ($p<0.05$), but this observed decline in GPx activity was significantly inhibited in the groups treated with cabbage leaf juice, wild honey and their combined form ($p<0.05$). This protective effects of the juice and honey were also observed in other antioxidant parameters such as superoxide dismutase (SOD), catalase (CAT) and MDA (Table 4.7 and Fig. 4.6).

Conclusion

The mixture of cabbage leaf juice and Honey has an effective ingredient, which can protect an individual from gastric ulcers. The combination of both had been tested in rats and can also be used for patients with ulcers in hospital and evaluates their nutritional status.

Recommendations

Specifically, ulcer in the stomach refers to gastric ulcer. The most frequent site of ulcer in the stomach is the lesser curvature, however, it can also occur anywhere from pylorus to cardia. The standardization of honey in concentration plays the important bench mark as a researcher, one must make sure that the honey is pure and not adulterated. The source of the cabbage leaf is important as well as the identification of the leaf by a botanist. The leaf must be properly washed to avoid contamination.

References

Agrawal, A. K., Rao, C. V., Sairman, K., Joshi, V. K and Goel, R. K., (2000). Effect of piper lon gum linn, *Zingiber officionial linn* and *Ferula* species on gastric ulceration and secretion in Rats. *J. Exp. Bio*. 38(10): 994- 998

Ali, A. T and Al-swayeh, O. A (2003). Honey potentes the gastric protection effects of sucraffate against ammonia induced gastric lesions in rats, *Saudi J. Gastroenterol.* 9: 117 – 123.

Al-mazrooa, A., Dat fresi and Mansoor Suleiman (1999). Effect of honey on stress induced ulcers in rats. *J. Kau. Med. Sci.* 7(1): 115 -122.

Barchi, A., Miraglia, C., Violi, Cambie, G., Noverine, N., Capsso, M., Leandro, G., meschi, T. De'Angelis ,G.L., Di-Merio,F.A. (2018), non-bivasive method for the diagnosis of upper GI diseases ACTA Biomed.89 (8): 40 – 43.

Bukhari,M. H., Javed, K., Samina, Q., Zahid, Q., Muhammed, Z., Navid, A., and Irfan, M. B.,

(2011). Comparative gastroprotective effects of natural honey, nigella sativa and gimetidine against cetylsalicylic acid induced gastric ulcer in albino rats. *J. of the coll. Of Physicians and Surgeons Pakistain.* 21 (13): 151 -156.

Jarosz, M., Dzienizewski, J., Dabrowska-ufniarz, Wartano-wicz, M., Ziemlanski, S., Reed, P.I. , (1998). Effect of high dose vitamin C treatment in *Helicobacter phlori* infection and total vitamin C concentration in gastric juice. *Eur.J.,Cancer . Prev.* 7(6): 449 – 454.

Kayali, S., Manfredi, M., Gevani, F., Bianchi, S (2018). *Helicobacter pylori* transmission routes and recurrence of infection. Acta Biomed. 89: 72 – 76

Loana, A. C and Andrea, Pop-Crison (2022). Correlation between endoscopic and Histopathological Assessment of *Helicobacter pylori* induced gastric pathology A cross – sectional retrospective study. *National Library of Medicine* 12 (12): 2096 – 2099.

Matong, F., Nwodo, U. U., (2014). In vitro assessment of *Helicobacter pylori* Ureases inhibition by honey fractions. *Arch Med. Res.* 45: 540 – 546.

Muriel, P. B.,Joao, P. B., Jairo, K. B., and Sergis, F. A., (2017). Effect of Brazilian green propolis on experimental gastric ulcers in rats, *J. ethnopharmacology*: 110 (3): 567 – 571

Pillai, S. I., Kandaswany, M., and Subramanian, S., (2010). Antiulceragenic and Ulcer healing effects of India propolis in experimental rats ulcer models. *International bee research Association* 2(1): 21.